Supplemental Experimental Procedures

Bone marrow derived dendritic cells (BMDCs)

All animal protocols were reviewed and approved by the MIT / Whitehead Institute / Broad Institute Committee on Animal Care (CAC protocol 0609-058-12). To obtain sufficient number of cells, we implemented a modified version of the DCs isolation protocol as previously described (Amit et al., 2009; Chevrier et al., 2011; Garber et al., 2012; Lutz et al., 1999). Briefly, for all CRISPR knockout experiments six- to eight-week old constitutive Cas9-expressing female mice were used as described previously (Platt et al., 2014). For all other experiments C57BL/6J female mice were obtained from the Jackson Laboratories. RPMI medium (Invitrogen) supplemented with 10% heat inactivated FBS (Invitrogen), β-mercaptoethanol (50μM, Invitrogen), L-glutamine (2mM, VWR), penicillin/streptomycin (100U/ml, VWR), MEM non-essential amino acids (1X, VWR), HEPES (10mM, VWR), sodium pyruvate (1mM, VWR), and GM-CSF (20 ng/ml; Peprotech) was used throughout the study.

Primary and Secondary Screen.

At day 0, cells were collected from femora and tibiae and plated in 100mm non tissue culture treated plastic dishes using 10ml medium per plate at concentration of 2×10^5/ml. At day 2, cells were fed with another 10ml of medium per dish. At day 5, 12ml of the medium were carefully removed (to avoid removal of cells) and 10ml of fresh medium were added back to the original dish. Cells were fed with another 5ml medium at day 7. At day 8, all non-adherent and loosely bound cells were collected and harvested by

centrifugation. Cells were then re-suspended with medium, plated at a concentration of $10x10^6$ cells in 10ml medium per 100mm dish. At day 9, cells were stimulated for various time points with LPS (100ng/ml, rough, ultrapure *E. coli* K12 strain, Invitrogen) and harvested.

Volumes were adjusted in proportion for different sized plates or wells (e.g. 96 well plates), but cells were always plated at concentration of 2×10^5/ml at day 0.

Individual sgRNA CRISPR knockout experiments

Individual sgRNA mediated CRISPR knockout experiments were performed as described previously (Platt et al., 2014). Briefly, BMDCs were isolated and grown as described above, but in addition were infected with lentiviruses encoding sgRNAs of interest at high MOI at day 2. Cells were expanded in the presence of GM-CSF. At day 6, infected cells were selected by adding puromycin (Invitrogen) at 5 μg/ml. At day 9, cells were stimulated with LPS for the appropriate time and harvested. For subsequent antibody staining (*e.g.*, anti-Tnf), cells were stimulated with 100 ng/ml LPS (or 20 ng/ml for a number of potential negative regulators; see **Results**) and after 30min Brefeldin A (GolgiPlug™, BD Biosciences) was added to trap secreted protein within the cells. 8h post LPS stimulation the cells were harvested, fixed and stained.

Virus production

To produce lentivirus for the screen, we used the GeCKOv2mouse library in the lentiGuide-Puro vector (Sanjana et al., 2014). 10cm plates of 70% confluent 293T cells were transfected with 9 μg of the plasmid library, 9 μg of PAX2 vector (Addgene) and

0.9 μg pVSVg using Lipofectamine® LTX and plus reagents according to the manufacturer's instructions. Supernatant was collected after 48 and 72 hours and then spun for 10 min at 4°C (3000 RPM) and then filtered with a 0.45μm membrane (PALL) and concentrated using Millipore® Amicon® Ultra-15 Centifugal Filter (40 min at 4°C at 4000 RPM). The virus was aliquoted and frozen at -80°C. The titer of the virus was determined by using BMDC from C57BL/6 mice followed by puromycin selection.

To produce lentiviruses containing individual sgRNAs for all validation and follow-up experiments, we used 96 well plates, analogous to the way described above, but with 1% of the reagents, without filtering or concentrating the virus. 20μl of the virus was then used to infect cells in each well of a 96 well plate and 200 μl to infect cells in each well in 12 well plates (in both cases the BMDCs were derived from Cas9 expressing mice). .

Fluorescent cell staining and FACS

For the pooled genome-wide and secondary CRISPR screens, BMDC activated with LPS in the presence of Brefeldin A were harvested on ice by scraping, washed twice with cold PBS, and fixed in 4% formaldehyde (Thermo Scientific) for 10 minutes at room temperature. After a further PBS wash, cells were washed with PBS containing 0.1% saponin (Sigma) and resuspended in PBS containing 0.1% saponin supplemented with the following fluorescent antibodies: eBioscience 12-7321-81 Anti-Mouse TNF alpha PE, Biolegend 117309 APC anti-mouse CD11c Antibody diluted 1:200. After an incubation of 30 minutes on ice, the stained cells were washed once with PBS containing 0.1% saponin, and twice with PBS before sorting.

FACS sorting was performed at the Bauer Core Laboratory, Harvard FAS Center for Systems Biology, Cambridge, MA. In two out of the three replicates of the screen, Cd11c+ cells were sorted into three bins. Two bins had low Tnf expression, to capture cells containing sgRNA targeting positive regulators. The third bin collected the highest 5% of Tnf-expressing cells. In the first experiment, the cells were sorted into two bins (low and high). The bin boundaries were guided by our observations of Tnf expression in cells infected with sgRNAs targeting the known regulators Tlr4, Myd88, and Zfp36 (**Figure 1A**).

For non-pooled CRISPR knockout experiments with individual sgRNAs, cell harvesting and staining was as described above, with the following modifications. Because of cell death caused by puromycin selection of lentivirus-infected cells, dead cells were labeled prior to fixation with Fixable Viability Dye eFluor® 520 (eBioscience) in the majority of experiments, following the manufacturer's instructions. In these experiments, each sample was divided in half after fixation and stained with two antibody panels. Both panels contained Biolegend 117326 PerCP anti-mouse CD11c Antibody. Panel 1 additionally contained Tnf-PE as described above and eBioscience 17-0141-81 Anti-Mouse CD14 APC. Panel 2 additionally contained R&D Systems IC450P Mouse CCL3/MIP-1 alpha Phycoerythrin mAb, and Biolegend 504508 APC anti-mouse IL-6 Antibody. In a minority of experiments, cells were stained with only one panel, in the same way as for the screen, with the addition of eBioscience 11-0141-82 Anti-Mouse CD14 FITC. All antibody dilutions were 1:200, except anti-CCL3/Mip-1 alpha, 1:20.

Flow cytometry was performed on a BD Accuri C6 cytometer in 96- well plates. Analysis was done in FlowJo (Treestar).

In non-pooled CRISPR knockout experiments with individual sgRNAs, low cell growth or viability could be caused by sgRNA-mediated mechanisms, or by low lentiviral titer. To distinguish between these, we separately transduced BMDC from Cas9 mice and C57BL/6 mice (which do not express Cas9). On day 9 of cell growth we harvested the cells and measured viability with Fixable Viability Dye eFluor® 520 followed by flow cytometry analysis, as described above. An sgRNA was considered to cause a cell growth or viability phenotype if the proportion of live cells in Cas9-expressing cells was reduced compared to the proportion of live cells in the C57BL/6 cells. Based on these experiments, we excluded several genes that reduce viability as well as any guides that did not show a consistent phenotype (**Table S2**).

DNA purification from infected cells and library preparation to determine which sgRNAs were expressed in the infected DCs

DNA was purified using Qiagen DNeasy Blood & Tissue Kit according to the manufacturer's instruction. Briefly, the cross-linked cells were first treated with Proteinase K and incubated at 55C for 4h to de-crosslink the DNA. DNA was purified according to the kit's protocol and eluted in 400 µl H_2O. We performed two successive PCR reactions of 20 cycles each as described previously (Shalem et al., 2014) using Herculase II Fusion DNA Polymerase (Agilent). 15µl from the first PCR was used for the

second PCR (100µl) primers included barcodes as described (Shalem et al., 2014). The final PCR product was run on a gel and the right size fragment was gel extracted and sequenced on a Hi-seq 2500. On average, we sequenced 4-6 aligned reads per sorted cell in each of the bins.

Cloning individual sgRNAs

Pairs of oligonucleotides (IDT) with BsmBI-compatible overhangs were separately annealed and ligated to lentiGuide-Puro plasmid (also available at Addgene, plasmid # 52963) using standard protocols. sgRNA target sequences were taken from the GeCKO library (Sanjana et al., 2014) to validate screen results, or were generated using a previously described sgRNA design algorithm (Doench et al., 2014) (**Table S5**). Oligonucleotide pairs were designed as follows:

Forward: 5' CACCG<sgRNA target sequence> 3'

Reverse: 5' AAAC<sgRNA target reverse complement>C 3'

In addition, for the initial calibration experiment (**Figure 1A**) we used the following sgRNAs:

Myd88 5' CCCACGTTAAGCGCGACCAA 3'

Zfp36%%MGLibA_60687 5' GGATCTCTCTGCCATCTACG 3'

Tlr4%%MGLibA_54042 5' GATCTACTCGAGTCAGAATG 3'

NonTargeting 5' GGGGTAGGCCTAATTACGGA 3'

Design and cloning of the secondary library

For the secondary screen, we targeted the top 2,569 genes in the DE analysis of the primary screen. We used the method of (Doench et al., 2014) to design 10 sgRNAs per gene and included another 2,500 non targeting sgRNAs (**Table S5**). For library construction we used a previously published protocol (Shalem et al., 2014). Briefly, synthesized oligos (Broad Technology Labs) were amplified using the following primers Forward TAACTTGAAAGTATTTCGATTTCTTGGCTTTATATATCTTGTGGAAAGGAC GAAACACCG Reverse ACTTTTTCAAGTTGATAACGGACTAGCCTTATTTTAACTTGCTATTTCT AGCTCTAAAAC and cloned to BsmBI (Fermentas) digested lentiGuide-Puro plasmid (also available at Addgene, plasmid # 52963) using Gibson ligation reaction (NEB). The ligation reaction was performed using molar ratio of 1:5 of the vector to insert.

Electrocompetent Endura™ Competent Cells (Lucigen) were transformed with the products of the ligation reaction according to the manufacturer's protocol using a GenePulser (BioRad). 10 parallel transformations were performed and plated onto 245 mm x 245 mm plates with carbenicillin selection (100 ug/ml), for 16 hours at 32 degrees. Colonies were collected and plasmid DNA extraction was made using Endotoxin-Free Plasmid Maxiprep (Qiagen). Virus production and all subsequent steps were performed as described for the primary screen. Cells were sorted to "low" and "high" TNF bins. The low TNF bin contained more cells and was further divided to three equal aliquots for

library preparation. These three "low" TNF libraries where treated as technical replicates for the DE analysis, and averaged for the Z score analysis (see below).

sgRNA sequence analysis

Raw sequencing reads were converted to FASTA files using fastq_to_fasta (FASTX-Toolkit http://hannonlab.cshl.edu/fastx_toolkit/), sequences flanking the guides sequence were trimmed using cutadapt-1.4.1 (Martin 2011) and the trimmed reads were aligned to the sgRNA sequences in the plasmid library using Bowtie 1 (Langmead et al., 2009), with no mismatches allowed. To exclude lowly abundant sgRNAs, the lowest 5% quantile of guides in the input lentiviral libraries was removed from all samples.
In the last two replicate experiments of the screen, we found sgRNA contamination that resulted from individual sgRNA cloning of the following guides: Tlr4%%MGLibA_54042, Tlr4%%MGLibB_54025, Dgke%%MGLibB_14010, Dgke%%MGLibA_14019, Rab13%%MGLibB_44085, Rab13%%MGLibA_44099, Tnf%%MGLibB_55164, Tnf%%MGLibA_55183. Those guides were discarded from our analysis.

Since the C57BL/6 genome was the template for the sgRNA, we assessed the possibility that any sgRNA overlapped a single nucleotide polymorphism (SNP) in two additional genomes that contributed to the CAS-9 transgenic mice. Specifically, The CAS-9 mice were created using 3 different mouse strains, C57BL/6, 129 and FVB. In order to examine possible effects of single nucleotide polymorphism (SNP) between the strains on the sgRNA efficiency, we compared the coordinates of the SNP data

(http://www.sanger.ac.uk/resources/mouse/genomes/) to the genomic mappings of the sgRNA sequences. Of 119,364 sgRNA sequences perfectly and uniquely matching the mm10 genome, only 3,350 sgRNA contained one SNP to one of the other two genomes, 255 sgRNAs contained 2 SNPs and 34 sgRNAs contained 3 or more SNPs.

Scoring sgRNA enrichment or depletion by differential expression analysis

To score sgRNAs whose levels are distinct between the TNF^{low} and TNF^{hi} cells, we used a differential expression analysis. First, we combined the two TNF^{low} bins by averaging for each sgRNA its read counts in the two bins. Next, we performed differential expression analysis on three biological repeats of TNF^{low} and TNF^{high} bins using the R package DESeq2 (Love et al., 2014) that fits a negative binomial generalized linear model (GLM). Normalization factors were provided to DESeq2 using non-parametric quantile normalization (R package EDASeq, 'betweenLaneNormalization'). Significant differences in abundance in TNF^{low} versus TNF^{high} bins were tested using a likelihood ratio test, testing the difference in deviance between a reduced model: counts ~ experiment and a full model: counts ~ experiment + TNF^{low}/TNF^{high}.

For the secondary library, the default size factor normalization of DESeq2 was used and differential expression was tested using a Wald test.

Scoring sgRNA enrichment or depletion by Z-score analysis

As a second strategy to score sgRNAs whose levels are distinct between the TNF^{low} and TNF^{hi} cells, we used a Z-score based approach. First, we added one read to all the

samples, and then quantile normalized the samples. We combined all low and high bins from the three experiments, into a single pair of TNF^{low} and TNF^{hi} bins, using the geometric mean of the quantile-normalized values. We performed the same procedure on all post-LPS, pre-LPS, and Input libraries. To control for the correlation of fold change to mean abundance, fold changes of TNF^{low} / TNF^{hi} were standard normalized in 12 bins of mean abundance (mean of TNF^{low} and TNF^{hi}), each containing ~10,000 guides. To collapse to gene level, the mean of the top four ranked sgRNAs was taken for positive regulators, and the bottom four ranked sgRNAs for negative regulators. Empirical *P*-values were calculated by randomly assigning sgRNAs to genes and false discovery rates (FDR) were assessed using the method (Benjamini and Hochberg 1995).

For the secondary library, we normalized each sample by the total number of reads and multiplied by 10^6. Because the secondary library is enriched for regulators, the fold changes of TNF^{low} / TNF^{hi} were standard normalized with respect to the non-targeting guides within each window (6 windows in total). We then averaged on all sgRNAs per gene, the non-targeting guides were randomly collapsed to “genes” (10 sgRNAs per “gene” to mimic the analysis for the targeting sgRNAs). The ranking in the secondary screen section in the **Results** is based on Z score of positive regulators, the FDR include the positive and negative regulators.

In both analysis methods we include only genes that had 4 or more sgRNAs that pass the abundance filter.

1471 sgRNAs perfectly mapped to more than one gene. 1302 genes are affected by this redundancy. Of these, for 434 genes, all sgRNAs map to an indistinguishable member(s) of a paralogous gene family. We report arbitrarily one of the genes names in **Tables S1-S4** and report the 434 genes names in **Table S5**.

For the remaining 868 genes, at least one sgRNA is multiply-mapped, leading to potential loss of sensitivity and confounding effects. We report these genes in **Table S5.**.

Classification of known genes that have immune annotation

We analyzed five major databases of immune gene annotations: Immport (Bhattacharya et al., 2014), IRIS (Kelley et al., 2005), Immunome (Ortutay and Vihinen, 2006), MAPK-NFkB network (Lynn et al., 2008), and the TLR pathway as defined by KEGG (Kanehisa and Goto, 2000). Genes that are not included in any of those databases (**Table S1**) were related as "new" or "not previously annotated".

To define a core gene set known to regulate our model system, we chose the subset of KEGG's TLR pathway genes that can directly connect LPS to Tnf in the pathway map found at this link: http://www.genome.jp/kegg-bin/show_pathway?mmu04620. This gene set was used for **Figure 1E** and **Figure 2B**.

Identifying significant effects on protein expression

To assess the impact on marker protein expression by individual sgRNAs and the genes they target we used the following procedure. First, for each protein marker and sgRNA,

we tested for a significant difference between the distribution of protein expression (from flow cytometry) measured for the sgRNA and the distribution of protein expression from all non-targeting sgRNAs within the same plate, using a KS-test and reporting the KS-statistic, D_n. We then signed the KS-statistic D by a one-sided Wilcoxon rank sum test. For each non-targeting sgRNA we determined an individual KS-statistic D_n using all non-targeting sgRNAs within the same plate as described above. To control for any plate effects, the KS-statistics within each plate were further standard normalized using the mean and standard deviations of the D (KS-statistic) values calculated for the non-targeting sgRNAs in that plate. Samples that had a cell count lower than 1,500 were excluded from further downstream analysis.

Next, we collapsed biological repeats of the same sgRNA using the mean of the Z-scored KS-statistic. High quality plates that included live / dead staining were favored over other plates; only when no measurement in the high quality plates was available for a given sgRNA, the measurements of the low quality plates were included. A tested sgRNA was considered a true positive regulator of Tnf if it passed the cutoff of -1.5 Z-score, which was determined using the Tnf Z-scores of the non-targeting sgRNAs.

Finally, true positive guides were collapsed to genes by taking the mean of the Z-scored KS-statistic across the sgRNAs, again favoring the high quality plates as we did for collapsing biological repeats of the same guide. For **Figure 3a** and **Figure S3A**, we conservatively excluded genes if one sgRNA targeting the gene showed a significant effect on a marker (cutoff -1.5 Z-score) and the other sgRNA did not, and the absolute Z-

score difference was >2.5. Five genes were filtered due to such discrepancies (Tnf, Gpkow, Pabpc1, Map3k8, Srpr).

Note, that since the distribution can be multi-modal and skewed in varied ways, we also manually visually inspected – independently and blindly to the computational analysis – the distributions in each individual experiment and each individual marker. There were four discrepancies between the automated and manual calls (Traf6, Akrin2, Ddx39b), and they therefore are not presented in **Figure 3A**.

RNA-Seq

BMDCs were infected with individual sgRNAs, expanded and differentiated in the presence of puromycin (from day 6 onward) in 96 well plates. At day 9, LPS was added for 2, 4 or 6 hours with or without Brefeldin (or not added at all – time point 0h). RNA was purified using Qiagen RNAeasy 96 Kit according to the manufacturer's instructions. The RNA was eluted in a volume of 50µl. For library construction we used the SMART-seq2 protocol (Picelli et al., 2013) in a 96 well plate format and with several modifications. 2 µl of RNA sample per well were mixed with 2µl RT primer (10µM 5´-AAGCAGTGGTATCAACGCAGAGTACT30VN-3´), 2µl dNTP mix (10mM each, Agilent Technologies) and 2µl Recombinant RNase Inhibitor (RRI-Clontech). This mix was incubated for 3min at 72°C and immediately placed on ice. To perform reverse transcription (RT) we added a mix of 1.5µl H2O, 4 µl Maxima buffer (ThermoFisher Scientific), 4 µl Betaine (5M SIGMA-ALDRICH), 1.8 µl MgCL2, 2µl TSO (10µM AAGCAGTGGTATCAACGCAGAGTACrGrG+G), 0.5 RRI and 0.2µl Maxima H

Minus Reverse Transcriptase enzyme (ThermoFisher Scientific). We incubated the RT reaction mix at 42°C for 90 min followed by 10 cycles of 50°C for 2 min, 42°C for 2 min, afterwards heat inactivated the enzyme for 15 min at 70°C. We used 11µl of the RT reaction for the PCR reaction by adding 12.5 µl KAPA HiFi Hotstart (KAPA Biosystems), 1µl H2O and 0.5 µl of (10µM 5'-AAGCAGTGGTATCAACGCAGAGT-3′) primer under the following conditions: 98°C for 3 min, 14 cycles of (98°C for 15 sec, 67°C for 20 sec, 72°C for 6 min), final extension at 72°C for 5 min. The PCR product was used for library preparation with Nextera XT DNA Sample Preparation (Illumina) according to the manufacturer's instructions. Samples were combined and purified using Ampure XP Agencourt beads (Beckman Coulter) and sequenced on a Hi-Seq 2500 (Illumina), to generate paired-end 25bp reads. Each sample was sequenced to an average depth of four million reads (IQR-2.3 -5.5 million).

RNA-seq analysis

We created a Bowtie index based on the mm9 mouse reference genome, and then aligned paired-end reads directly to this index using Bowtie v 0.12.7 (Trapnell et al., 2009). Next, we ran RSEM v1.11 (Li and Dewey, 2011) with default parameters on these alignments to estimate expression levels. RSEM's gene level expression estimates (tau) were multiplied by 1,000,000 to obtain transcript per million (TPM) estimates for each gene. To transform expression levels to log-space, we took the log2(TPM+1). Sequencing libraries that correlated poorly (Pearson r<0.8) with the majority of samples or had fewer than 500,000 expected counts across the transcriptome were removed from further analysis.

The log2(TPM+1) values were then quantile normalized, which reduced the coefficient of variation for each gene across samples. Without batch correction, samples separated strongly according to the experiment and plate in the first two principal components within a given time point. Batch correction was performed using the SVA package in R (Leek et al., 2012) using ComBat (Johnson et al., 2007). The primary known batch covariate was the plate on which the sample was processed. After batch correction, the effects associated with batch were attenuated.

To determine mRNAs whose expression is affected by knockout of individual genes, we first collapsed expression profiles from multiple sgRNAs that target the same gene, as long as those sgRNAs had expression profiles that were significantly correlated. To determine with sgRNAs had significantly correlated profiles, we compared their pair-wise linear (Pearson) correlation to a background distribution of Pearson correlation coefficients between non-targeting sgRNAs and all other sgRNAs within a time point. We then averaged the batch-corrected log2(TPM+1) data for all sgRNAs targeting the same gene whose pair-wise correlations exceeded the threshold of one standard deviation from the background distribution. Finally, we Z-transformed the expression profiles (collapsed from guides to genes) relative to the expression values for non-targeting sgRNAs at the same time point. We set a threshold for significance of the effect of a perturbed gene on a target mRNA to a Z-score of four. We clustered the collapsed and Z-transformed profiles using hierarchical agglomerative clustering with complete linkage and a Pearson correlation (**Figure 3D**).

To identify genes that are differentially expressed between a set of experiments involving members of one module or complex vs. other modules or non-targeting controls (as per **Figure 4B-F**) we used differential expression analysis with the Wald test in the DESeq2 package (Love et al., 2014) with default parameters using the expected counts from RSEM. Batch correction was taken into account in the experimental design. Analysis of differential expression by complex/module was performed between the expression profiles from all samples transduced with sgRNAs targeting the complex to the expression of samples containing non-targeting sgRNAs collected at the same time point.

Paf1 and Rtf1 Immunopurification (IP) followed by quantitative mass spectrometry to identify interaction partners

For each IP 20 million unstimulated BMDCs (day 9) derived from C57BL/6J female mice were used. Paf1 IP was performed by using anti-Paf1 antibody (Bethyl Laboratories, A300-173A) and Rtf1 IP by using anti-Rtf1 antibody (Bethyl Laboratories, A300-178A). Control IPs were performed with a rabbit IgG control antibody (Bethyl Laboratories, P120-101). Each Paf1 or Rtf1 IP was always performed in parallel to a control IP and in two independent replicates.

BMDCs were harvested and washed twice with ice-cold PBS and lysed for 30 min in 400µl ice-cold lysis buffer (150 mM NaCl, 50 mM Tris/HCl pH 7.5, 1% IGPAL-CA-630 (Sigma, #I8896), 5% Glycerol, 2 µg/mL aprotinin (Sigma, A6103), 10 µg/mL leupeptin (Roche, #11017101001), 1 mM PMSF (Sigma, 78830)). Lysates were centrifuged at

14,000*g* for 10 min. In parallel, 100 µl of Protein G Dynabeads (Life Technologies) per IP were washed 3 times in 500 µl lysis buffer. Cleared lysate, washed Protein G Dynabeads and 10 µg of antibody were all mixed together in a 1.7 ml Eppendorf tube and incubated on a rotator at 4°C overnight (16-18 hours). After overnight incubation, the supernatant was removed, the beads washed twice with 500 µl ice-cold wash buffer (150 mM NaCl, 50 mM Tris/HCl pH 7.5, 5% Glycerol) + 0.05% IGPAL-CA-630 (Sigma, #I8896) and two additional times with 500 µl ice-cold wash buffer only. The beads were then incubated with 80 µl urea/trypsin buffer (2 M urea, 50 mM Tris/HCl pH 7.5, 1 mM DTT, 5 µg/ml Trypsin (Promega)) for 1 hour at 25°C on a shaker (1,000 rpm) in order to release the bound proteins by an on-bead protein digest. Next, the supernatant was transferred to a new Eppendorf tube and the beads were washed twice with 60 µl urea buffer (2 M urea, 50 mM Tris/HCl pH 7.5). The supernatant of the two washes and the on-bead digest were combined (total of 200 µl), centrifuged at 5,000g in order to remove residual beads and the supernatant was further processed for mass spectrometry.

Disulfide bonds were reduced with 5 mM dithiothreitol (DTT) and cysteines were subsequently alkylated with 10 mM iodoacetamide. Samples were further digested by adding 0.5 µg sequencing grade modified trypsin (Promega) at 25°C. After 16 h of digestion, samples were acidified with 1% formic acid (final concentration). Tryptic peptides were desalted on C18 StageTips according to (Rappsilber et al., 2007) and evaporated to dryness in a vacuum concentrator.

Desalted peptides of the first repeat of the IPs (replicate 1) were labeled with the iTRAQ reagent according to the manufacturer's instructions (AB Sciex) and as previously described (Mertins et al., 2012). Briefly, 0.5 units of iTRAQ reagent were used per IP. Peptides were dissolved in 15 µl of 0.5 M TEAB pH 8.5 solution and the iTRAQ reagent was added in 35 µl of ethanol. After 1 h incubation the reaction was stopped with 50 mM Tris/HCl (pH 8.0). Differentially labeled peptides were mixed and subsequently desalted on C18 StageTips (Rappsilber, Mann, and Ishihama 2007) and evaporated to dryness in a vacuum concentrator. Peptides were reconstituted in 10 µl 3% MeCN/0.1% formic acid. LC-MS/MS analysis was performed as previously described (Mertins et al., 2013).

Desalted peptides of the second repeat of the IPs (replicate 2) were labeled with the TMT10plex mass tag labeling reagent according to the manufacturer's instructions (Thermo Scientific) with small modifications. Briefly, 0.1 units of TMT10plex reagent was used per IP. Peptides were dissolved in 10 µl of 50 mM Hepes pH 8.5 solution and the TMT10plex reagent was added in 4.1 µl of MeCN. After 1 h incubation the reaction was stopped with 1 µl 5% Hydroxylamine for 15 min at 25°C. Differentially labeled peptides were mixed and subsequently desalted on C18 StageTips (Rappsilber et al., 2007) and evaporated to dryness in a vacuum concentrator. Peptides were reconstituted in 20 ul 3% MeCN/0.1% formic acid. LC-MS/MS analysis was performed as previously described (Mertins et al., 2013)..

All mass spectra were analyzed with MaxQuant software version 1.5.2.8 (Cox and Mann, 2008) using the mouse UniProt database (July 2014) (UniProt, 2015) MS/MS searches

for the proteome data sets were performed with the following parameters: Oxidation of methionine and protein N-terminal acetylation as variable modifications; carbamidomethylation as fixed modification. Trypsin/P was selected as the digestion enzyme and 2 missed cleavages per peptide were allowed. The mass tolerance for precursor ions was set to 20 p.p.m. for the first search (used for nonlinear mass re-calibration) and 6 p.p.m. for the main search. Fragment ion mass tolerance was set to 20 p.p.m. For identification, we applied a maximum FDR of 1% separately on the protein and peptide level. We required 2 or more unique/razor peptides for protein identification and a ratio count of 2 or more for protein quantification per replicate measurement in at least one of the two replicates.

We calculated for each protein the log2 ratio between each candidate IP (Paf1 or Rtf1) over its control IP (rabbit IgG) for each replicate independently. We then subtracted for each replicate and IP the median of the distribution of the log2 transformed values (across all proteins that passed our filter: quantified in both replicates and in at least one replicate by two or more unique/razor peptides for protein identification and a ratio count of 2 or more for protein quantification) from the individual log2 ratios of each protein, to center the log2 ratio distribution around 0. Proteins with a log2 ratio > 0.8 (> 1.7 fold) in both replicate IPs were considered to be interactors.

Verification of the Paf1 and Auh interaction by Western blot

We performed Western plot on the cleared cell lysate (=Input) and the Protein G dynabeads after overnight incubation with the cell lysate and either Paf1 antibody or

control rabbit IgG and the subsequent four washes (IP). We used anti-Paf1 antibody (Bethyl Laboratories, A300-173A) and anti-Auh antibody (ABCAM, ab155980).

DNA sequencing of cut site

To quantify the fraction of sequencing reads that reflect loss-of-function alleles we examined their alignments to the genomic target region. First, we excluded contaminating reads that do not have an exact match to at least one 20bp segment in the genomic target region. Second, we used Smith-Waterman local alignment and identified all mismatches, insertions and deletions (indels) in the read relative to the aligned portion of the genomic target region. We focused on indels, since the effect of mismatches on protein function is not easily predicted. We then calculated the combined length of the shift due to the remaining indels, *i.e.*, the total length of insertions minus the total length of deletions. Note that most reads contain only a single indel. All reads with indels whose combined length is not a multiple of three were defined as frame-shift reads and thus loss-of-function alleles, while all other non-excluded reads were defined as functional alleles.

Accession numbers

The RNA-Seq data is deposited in the Gene Expression Omnibus accession number GSE67164. The sgRNA sequencing data is deposited in

http://www.broadinstitute.org/pubs/TNF_CRISPR_DCs/

The processed mass spectrometry data is reported in **Table S4** and raw mass spectrometry data is available upon request.

Supplemental References

Amit, I., Garber, M., Chevrier, N., Leite, A.P., Donner, Y., Eisenhaure, T., Guttman, M., Grenier, J.K., Li, W., Zuk, O., *et al.* (2009). Unbiased reconstruction of a mammalian transcriptional network mediating pathogen responses. Science *326*, 257-263.

Benjamini, Y., and Hochberg, Y. (1995). "Controlling the false discovery rate: a practical and powerful approach to multiple testing". Journal of the Royal Statistical Society, Series B *57* (1): 289–300.

Bhattacharya, S., Andorf, S., Gomes, L., Dunn, P., Schaefer, H., Pontius, J., Berger, P., Desborough, V., Smith, T., Campbell, J., *et al.* (2014). ImmPort: disseminating data to the public for the future of immunology. Immunologic research *58*, 234-239.

Chevrier, N., Mertins, P., Artyomov, M.N., Shalek, A.K., Iannacone, M., Ciaccio, M.F., Gat-Viks, I., Tonti, E., DeGrace, M.M., Clauser, K.R., *et al.* (2011). Systematic discovery of TLR signaling components delineates viral-sensing circuits. Cell *147*, 853-867.

Cox, J., and Mann, M. (2008). MaxQuant enables high peptide identification rates, individualized p.p.b.-range mass accuracies and proteome-wide protein quantification. Nature biotechnology *26*, 1367-1372.

Doench, J.G., Hartenian, E., Graham, D.B., Tothova, Z., Hegde, M., Smith, I., Sullender, M., Ebert, B.L., Xavier, R.J., and Root, D.E. (2014). Rational design of highly active sgRNAs for CRISPR-Cas9-mediated gene inactivation. Nature biotechnology *32*, 1262-1267.

Garber, M., Yosef, N., Goren, A., Raychowdhury, R., Thielke, A., Guttman, M., Robinson, J., Minie, B., Chevrier, N., Itzhaki, Z., *et al.* (2012). A high-throughput chromatin immunoprecipitation approach reveals principles of dynamic gene regulation in mammals. Molecular cell *47*, 810-822.

Johnson, W.E., Li, C., and Rabinovic, A. (2007). Adjusting batch effects in microarray expression data using empirical Bayes methods. Biostatistics *8*, 118-127.

Kanehisa, M., and Goto, S. (2000). KEGG: kyoto encyclopedia of genes and genomes. Nucleic acids research *28*, 27-30.

Kelley, J., de Bono, B., and Trowsdale, J. (2005). IRIS: a database surveying known human immune system genes. Genomics *85*, 503-511.

Langmead, B., Trapnell, C., Pop, M., and Salzberg, S.L. (2009). Ultrafast and memory-efficient alignment of short DNA sequences to the human genome. Genome biology *10*, R25.

Leek, J.T., Johnson, W.E., Parker, H.S., Jaffe, A.E., and Storey, J.D. (2012). The sva package for removing batch effects and other unwanted variation in high-throughput experiments. Bioinformatics *28*, 882-883.

Li, B., and Dewey, C.N. (2011). RSEM: accurate transcript quantification from RNA-Seq data with or without a reference genome. BMC bioinformatics *12*, 323.

Love, M.I., Huber, W., and Anders, S. (2014). Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome biology *15*, 550.

Lutz, M.B., Kukutsch, N., Ogilvie, A.L., Rossner, S., Koch, F., Romani, N., and Schuler, G. (1999). An advanced culture method for generating large quantities of highly pure dendritic cells from mouse bone marrow. Journal of immunological methods *223*, 77-92.

Lynn, D.J., Winsor, G.L., Chan, C., Richard, N., Laird, M.R., Barsky, A., Gardy, J.L., Roche, F.M., Chan, T.H., Shah, N.*, et al.* (2008). InnateDB: facilitating systems-level analyses of the mammalian innate immune response. Molecular systems biology *4*, 218.

Martin, M. (2011). Cutadapt removes adapter sequences from high-throughput sequencing reads. EMBnet.journal 17, pp. 10–12.

Mertins, P., Qiao, J.W., Patel, J., Udeshi, N.D., Clauser, K.R., Mani, D.R., Burgess, M.W., Gillette, M.A., Jaffe, J.D., and Carr, S.A. (2013). Integrated proteomic analysis of post-translational modifications by serial enrichment. Nature methods *10*, 634-637.

Mertins, P., Udeshi, N.D., Clauser, K.R., Mani, D.R., Patel, J., Ong, S.E., Jaffe, J.D., and Carr, S.A. (2012). iTRAQ labeling is superior to mTRAQ for quantitative global proteomics and phosphoproteomics. Molecular & cellular proteomics : MCP *11*, M111 014423.

Ortutay, C., and Vihinen, M. (2006). Immunome: a reference set of genes and proteins for systems biology of the human immune system. Cellular immunology *244*, 87-89.

Picelli, S., Bjorklund, A.K., Faridani, O.R., Sagasser, S., Winberg, G., and Sandberg, R. (2013). Smart-seq2 for sensitive full-length transcriptome profiling in single cells. Nature methods *10*, 1096-1098.

Platt, R.J., Chen, S., Zhou, Y., Yim, M.J., Swiech, L., Kempton, H.R., Dahlman, J.E., Parnas, O., Eisenhaure, T.M., Jovanovic, M.*, et al.* (2014). CRISPR-Cas9 knockin mice for genome editing and cancer modeling. Cell *159*, 440-455.

Rappsilber, J., Mann, M., and Ishihama, Y. (2007). Protocol for micro-purification, enrichment, pre-fractionation and storage of peptides for proteomics using StageTips. Nature protocols *2*, 1896-1906.

Sanjana, N.E., Shalem, O., and Zhang, F. (2014). Improved vectors and genome-wide libraries for CRISPR screening. Nature methods *11*, 783-784.

Shalem, O., Sanjana, N.E., Hartenian, E., Shi, X., Scott, D.A., Mikkelsen, T.S., Heckl, D., Ebert, B.L., Root, D.E., Doench, J.G.*, et al.* (2014). Genome-scale CRISPR-Cas9 knockout screening in human cells. Science *343*, 84-87.

Trapnell, C., Pachter, L., and Salzberg, S.L. (2009). TopHat: discovering splice junctions with RNA-Seq. Bioinformatics *25*, 1105-1111.

UniProt, C. (2015). UniProt: a hub for protein information. Nucleic acids research *43*, D204-212.

Supplementary Figure Legends

Figure S1, related to Figure 1. Quality measures of a Genome wide pooled CRISPR screen in mouse primary DC. (**A**) Reproducibility. Shown are scatterplots comparing the $\log_2$(quantile normalized read counts) of sgRNAs between two replicate screens for the lowest bin (left), 2^{nd} lowest bin (middle), and top bin (right). Pearson correlation coefficient (*r*) is shown in top left corner. (**B-E**) Top ranked screen hits compare well between the DESeq and Z-score approaches. (B,C) Scatter plots compare the ranks based on the DE-Seq approach (X axis) and Z score approach (Y axis) for either positive regulators (B) or negative regulators (C) among the top-100 ranked genes. The Spearman rank correlation coefficient (ρ) is noted. (D, E) Shown is the Jaccard index between the Z-score and DE-Seq based approaches (Y-axis, intersection over union) for sliding windows of 50 genes from top of the ranked lists (X axis) for the true ranking (black) and with random shuffling (grey) of the Z-score ranks, for either the positive (D) or negative (E) regulators. The signal is diminished at rank ~150 and ~50 for positive and negative regulators, respectively. (**F**) sgRNAs that target translation genes are enriched in the "Input" library versus "Pre-LPS". Left: Scatterplot compares the normalized fold change in sgRNAs (Input / Pre-LPS) to the mean abundance in the two libraries. Middle and Right: Distribution of the normalized fold change (Input / Pre-LPS; Y axis) in either sgRNAs (middle) or genes (right; mean of the top 4 ranked sgRNAs). Orange: translation genes; black: all genes; grey: non-targeting controls. (**G**) sgRNAs targeting known regulators of LPS response are highly significant in DE-Seq analysis. MA-plots compare for either sgRNAs (left) or genes (right), the DE-Seq calculated fold-change between

TNF^{hi} and TNF^{low} (Y axis) to the mean abundance of the sgRNA or gene. (**H**) Screen hits are more likely to be expressed post-LPS and at higher level than all other genes. Violin plots show the distribution of mean expression (Y axis) along LPS stimulation (0h, 2h, 4h, 6h) in control cells, for top-169 hits (right) and for all other genes (left).

Figure S2, related to Figure 2. Assessing false negatives, negative regulators and analysis of the secondary library. Each panel in A-D shows flow cytometry staining of intracellular Tnf levels (X axis) for each targeted gene (colored histogram; gene name in top left corner) compared to sgRNA controls (black curves). (**A, B**) Determining the false negative rate. Known regulators of the LPS response that did not rank within the top-100 in the screen were tested individually by single sgRNAs followed by Tnf staining and flow cytometry (**Figure 2A**). (**A**) 8 tested genes did influence Tnf expression and are considered false negatives. (**B**) 6 tested genes did not influence Tnf expression and are considered true negatives. (**C-E**) Sensitive validation of novel negative regulators requires screening at unsaturated levels of Tnf. BMDCs transduced with single sgRNAs targeting candidate negative regulators from the screen, and stained with anti-Tnf antibody after stimulation with either **(C)** 100ng/mL LPS (two different sgRNAs shown for each gene) or **(D)** 20ng/mL LPS (single sgRNA shown for each gene). **(E)** BMDC transduced with an sgRNA targeting Stat5b were stained with Cd11c antibody. **(F-G)** sgRNAs targeting known regulators of LPS response (orange) have highly significant Z-scores, compared to other genes (black) and non-targeting controls (grey). Shown are MA-plots that relate for either sgRNAs **(F)** or genes **(G)** the z-score calculated fold-change between TNF^{hi} and TNF^{low} (Y axis) to the mean abundance of the sgRNA or gene

(X axis). **(H)** Top ranked screen hits compare well between the DE and ZS approaches. Scatter plot compares the ranks of each gene by the DE (Y axis) and ZS (X axis) approaches, for the top ranked 200 genes, of which they share 170. The Spearman rank correlation coefficient is noted at the upper left corner. **(I)** Secondary screen improves specificity. Shown are the theoretically estimated FDRs (Y axis), based on shuffling the guides before collapsing to genes, for the secondary screen (orange) and the primary screen (calculated as elsewhere based on top 4 sgRNAs, black; or according to all sgRNAs, grey). The empirical FDR for the first screen, as determined by validation experiment, is marked by light blue at all ranks up to 100.

Figure S3, related to Figure 3. Partitioning of the validated positive regulators into key modules by their effect on protein markers and RNA profiles and the affect of Brefeldin A. (**A**) Positive regulators group by distinct effects on protein expression. For each sgRNA targeting a positive regulator (rows) shown are its effects (Z score for each marker compared to non-target sgRNA; **Experimental Procedures**) on the expression of each of five proteins (columns) measured by staining with antibodies (**Experimental Procedures**). Three broad categories of responses can be defined, each preferentially associated with distinct proteins. Based on this matrix, sgRNAs were collapsed to score gene level effects as in **Figure 3A**. (**B**-**E**) Positive regulators partition to modules based on their effect on mRNA profiles over time. Shown are clustered correlation matrix of verified positive regulators (rows, columns) based on global RNA expression profiles in cells where the regulator is targeted relative to non-targeting control (**Experimental Procedures**). Data from each time point is analyzed and clustered (B) t=0h; (C) t=2h; (D)

t=4h; (E) t=6h). Genes in 3 key categories are color coded as in (A). Color bar is the Pearson correlation coefficient. Matrices are exactly as shown in **Figure 3D-F**, except that a matrix is also shown for t=6h, and that gene names are labeled. **F)** Effect of Brefeldin on the expression of the different modules. Violin plots show for each validated regulator (dot) the ratio of expression values (log(TPM+)) when comparing between Brefeldin *vs*. no Brefeldin conditions, in each of 3 modules and TNF (X axis).

Figure S4, related to Figure 4. Mutation analysis of sequencing sorted mutants and the affect of knockout OSTc subunits on different markers. BMDCs were transduced with sgRNA targeting the indicated gene ((**A**) Paf1, (**B**) Dad1 and (**C**) Cd14; marked on top), stimulated with LPS, and flow-sorted based on high or low Tnf antibody staining. Genomic DNA was isolated from sorted cells ("low Tnf" and "high Tnf"), unsorted cells ("Pre-sort"), and cells without relevant sgRNA (control; only in A and C). The region surrounding the sgRNA target site was amplified and sequenced to analyze mutational composition of the targeted locus. **D)** Each panel shows flow cytometry staining of the levels (X axis) of each of five protein markers (from left to right: Tnf, Cd11c, Cd14, Mip1α, Il6) in cells with individual sgRNA targeting specific genes (colored histogram; gene name in top left corner) compared to sgRNA controls (black curves). Data is shown (from top to bottom) for three representative members of OSTc (Ddost, Rpn1, Rpn2), and two other members of the module: Alg2 and Tmem258.

Figure S5, related to Figure 5. The PAFc module. (**A-D**) Each panel shows flow cytometry staining of either intracellular Tnf levels (A, B, D; X axis) or Cd11c levels (C,

X axis) for each targeted gene (colored histogram; gene name in top left corner) compared to sgRNA controls (black curves). (**E**) Validation of Paf1 and Auh interaction by Western blot. Shown are the immunopurifications (IPs) in BMDCs performed with either Paf1 antibody (PAF) or IgG antibody (Control). Input or IP samples were incubated with either Paf1 antibody (top) or Auh antibody (bottom). IPs were performed in unstimulated BMDCs (LPS "-") or in BMDCs stimulated with LPS for 2h (LPS "+ ").

Supplemental Table 1, related to Figure 1: Pooled screen and supporting data

Z-score ranks of genes depleted in "Pre-LPS" relative to "Input". The higher the rank (top genes) the more depleted in the "Pre-LPS" library (column B).

Z score analysis of the genome-wide screen. For positive regulators (column C), Z-score ranks of genes enriched in "Tnf^{lo}" relative to "Tnf^{hi}". The higher the rank the more enriched in the "Tnf^{lo}" library. For negative regulators (column D), Z-score ranks of genes depleted in "Tnf^{lo}" relative to "Tnf^{hi}". The higher the rank the more depleted in the "Tnf^{lo}" library.

Differential expression (DE) analysis of the genome-wide screen in "Tnf^{lo}" relative to "Tnf^{hi}". Rank (column E) based on the p-value (include positive and negative regulators). Standard DESeq output (columns F-K), including mean expression of all guides targeting each gene (column F) and the fold change (column G) between "Tnf^{lo}" and "Tnf^{hi}". Positive values indicate enrichment in the "Tnf^{lo}" library and therefore positive regulators. Negative values indicate depletion in the "Tnf^{lo}" library and therefore negative regulators.

Expression levels of top 169 candidates. Listed are the mean RNA expression values ($\log_2$(TPM+1)) of the 169 top candidate positive and negative regulators (column L). The expression value is the mean expression value of the gene in 24 non-targeting sgRNA controls at different time points post LPS activation from the RNA-seq samples.

RNA expression time course for the subset of the 169 top hits (35) whose expression is regulated more than 2 fold in the first 6h post LPS stimulation. The mean RNA expression values (log2(tpm+1)) for each time point in all non-Targeting control sgRNAs from the RNA-seq samples is given (columns M-P).

Summary of screen validation. Candidate positive (column Q) and negative (column R) regulators that were tested with individual sgRNA are noted. The number of sgRNA (in most cases 2-3 were tested) that verified the screen result for each gene are provided (columns S-U).

Immune annotation of validated positive regulators. Presence in five immune-related gene databases is noted by a value of "1" (columns V-Z). NI – the information for genes that are not validated positive regulators is not included.

Z score analysis of the secondary screen. Z score of "Tnf^{lo}" relative to "Tnf^{hi}"relative to the non-targeting sgRNAs (column AA) and computational false discovery rate (FDR) (column AB).

DE analysis of the secondary screen. Differential expression analysis of genes in the secondary library (standard DESeq output) between three technical replicate "Tnf^{lo}" (sorted cells divided to 3 sequenced separately) and one "Tnf^{hi}" (columns AC-AH).

Immune annotation of novel regulators based on ZS analysis of the secondary screen. Presence in five immune-related gene databases is noted by a value of "1" for the top 115 ranked genes from the secondary screen (columns AI-AM), including genes that were not scored as top ranked genes in the primary screen,. NI – the information for genes that are not relevant is not included.

Supplemental Table 2, related to Figure 2: Screen validation flow cytometry data

Guide-level data. All sgRNA for tested positive regulators are listed (column A). It is noted if expression of each guide leads to a significant loss in Tnf expression relative to control sgRNAs (column B), or was excluded from analysis due to one of three

considerations: Reduction of cell viability (column C), low lentiviral titer (column D), or irreproducible results (column E). Z-scores of intracellular staining levels of Tnf, Cd11c, Cd14, Mip1a, and IL6 relative to non-targeting control sgRNAs (columns F-J)..

Gene-level data. For all validated positive regulators, the flow cytometry staining based Z-scores of all tested protein markers relative to non-Targeting controls are given (columns P-T). The Z-scores are based on the value of the sgRNAs (see Experimental Procedure).

Supplemental Table 3, related to Figure 3: Differential expression analysis (DEseq) of RNA-seq data

Compares RNA expression in BMDC infected with the specified sgRNAs relative to expression in cells infected with non-targeting control sgRNAs in matched LPS treatment condition. Given is the base mean expression of each gene across samples, the log2 fold change between the group and the control group, the standard error of the log fold change lfcSE, the test statistic, p-values and adjusted p-values. The sgRNA and LPS treatment are as follows: sgRNA targeting Akirin, Pol2rg and Pabpc1 with LPS 2 hours (columns B-G), sgRNA targeting OSTc members (Rpn1, Rpn2, Ddost, and Dad) with no LPS treatment (columns H-M), sgRNA targeting Tmem258 with no LPS treatment (columns N-S).

Supplemental Table 4, related to Figure 5: Paf1 and Rtf1 immunoprecipitation

Shown are the normalized log2 ratios between the Paf1 IP over its control IP (rabbit IgG) of all proteins quantified in both replicates (columns B,C) and the log2 ratios between the

Rtf1 IP over its control IP (rabbit IgG) of all proteins quantified in both replicates (columns D,E).

The sheets “raw data Paf1 IP repl01 and 02” and “raw data Rtf1 IP repl01 and 02” show the relevant columns from the Maxquant out file “ProteinGroups”. The data presented is not filtered, but contaminant and reverse hits were removed, and the values shown are from the direct output of Maxquant. The columns presented are “Protein IDs”, which are the Uniprot identifier(s) of protein(s) contained in the protein group; “Majority protein IDs”, which are the Uniprot IDs of those proteins that have at least half of the peptides that the leading protein has; their associated “protein names”; “gene names”; “fasta header” in the search file; the “intensity”; “intensity not corrected”; and “ratio count”, which are the number of times peptides matching to the protein group were quantified and used to determine the protein signal intensity. “intensity”; “intensity not corrected”; and “ratio count” are shown for each IP performed (Paf1, Rtf1, Control (rabbit IgG); replicate 1 and 2 for each. Moreover “intensity”; “intensity not corrected”; and “ratio count” represent 4-plex iTRAQ channel values for replicate 1 and TMT 10-plex channel values for replicate 2. To calculate for each protein the log2 ratio between each candidate IP (Paf1 or Rtf1) over its control IP (rabbit IgG) for each replicate, “intensity” column values were used. Protein groups were only considered if they were detected and quantified in both replicate IPs and in at least one replicate by a “ratio count” of 2 or more for protein quantification (see Extended Experimental Procedure).

Supplemental Table 5, related to experimental procedures: sgRNA target sequences

sgRNA guide sequences that were generated using a previously described sgRNA design algorithm (Doench et al., 2014) to test knockout of genes that did not score high in our genome wide screen (columns A,B). List of sgRNA that were used in the secondary screen (columns D,E). F) Genes that overlap to other genes in all their sgRNA. G) Genes that contain overlap in some of their sgRNAs to other genes.